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STUDY PROTOCOL

The influence of human genetic variation on early transcriptional responses and protective immunity following immunization with Rotarix vaccine in infants in Ho Chi Minh City in Vietnam: A study protocol for an open single-arm interventional trial [version 1; peer review: 1 approved with reservations]

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Abstract

Background: Rotavirus (RoV) remains the leading cause of acute gastroenteritis in infants and children aged under five years in both high- and low-middle-income countries (LMICs). In LMICs, RoV infections are associated with substantial mortality. Two RoV vaccines (Rotarix and Rotateq) are widely available for use in infants, both of

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which have been shown to be highly efficacious in Europe and North America. However, for unknown reasons, these RoV vaccines have markedly lower efficacy in LMICs. We hypothesize that poor RoV vaccine efficacy across in certain regions may be associated with genetic heritability or gene expression in the human host.

Methods/design: We designed an open-label single-arm interventional trial with the Rotarix RoV vaccine to identify genetic and transcriptomic markers associated with generating a protective immune response against RoV. Overall, 1,000 infants will be recruited prior to Expanded Program on Immunization (EPI) vaccinations at two months of age and vaccinated with oral Rotarix vaccine at two and three months, after which the infants will be followed-up for diarrheal disease until 18 months of age. Blood sampling for genetics, transcriptomics, and immunological analysis will be conducted before each Rotarix vaccination, 2-3 days post-vaccination, and at each follow-up visit (i.e. 6, 12 and 18 months of age). Stool samples will be collected during each diarrheal episode to identify RoV infection. The primary outcome will be Rotarix vaccine failure events (i.e. symptomatic RoV infection despite vaccination), secondary outcomes will be antibody responses and genotypic characterization of the infection virus in Rotarix failure events.

Discussion: This study will be the largest and best powered study of its kind to be conducted to date in infants, and will be critical for our understanding of RoV immunity, human genetics in the Vietnam population, and mechanisms determining RoV vaccine-mediated protection.

Registration: ClinicalTrials.gov, ID: [NCT03587389](https://clinicaltrials.gov/ct2/show/study/NCT03587389). Registered on 16 July 2018.

Keywords

Rotavirus, Vaccine, Gene expression, Antibody response, Infants, Randomized controlled trial.



This article is included in the [Oxford University Clinical Research Unit \(OUCRU\) gateway](#).

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[report](#)

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Any reports and responses or comments on the article can be found at the end of the article.

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Abbreviations

RoV: Rotavirus

LMICs: Low-middle income countries

EPI: Expanded Program on Immunization

G: Glycoprotein

P: Protease-sensitive protein

GSK: GlaxoSmithKline

GWAS: Genome wide association studies

SNP: Single-nucleotide polymorphisms

WHO: World Health Organization

HCMC: Ho Chi Minh City

HVH: Hung Vuong Hospital

RNA: Ribonucleic acid

EDTA: Ethylenediaminetetraacetic acid

DNA: Deoxyribonucleic acid

HTD: Hospital for Tropical Diseases

AE: Adverse event

SAE: Serious adverse event

SUSAR: Suspected unexpected serious adverse reaction

OUCRU: Oxford university clinical research unit

CTU: Clinical trials unit

HLA: Human leukocyte antigen

CRF: Case report form

Background

Diarrhoea is the second leading cause of childhood morbidity and mortality globally¹, with 27% of all diarrhoea-related deaths associated with rotavirus (RoV) infection². RoV is the single most important cause of acute gastroenteritis in infants and children under 5 years of age in high- and low-middle-income countries (LMICs)². At the turn of the millennium, RoV was responsible for >125 million episodes of gastroenteritis and over 400,000 deaths per year^{3,4}. Although the median age of primary RoV infection is generally lower in developing countries (6–9 months) as compared to developed countries (2–5 years), nearly every child globally has been infected with RoV at least once by 5 years of age³. RoV belongs to the genus *Reoviridae* and is currently segregated into seven groups (A–G); Group A is the most dominant cause of gastroenteritis in humans⁵. Consequently, Group A RoVs are further characterized into genotypes according to variability in their glycoprotein (G) and protease-sensitive protein (P)^{5,6}. Due to high genetic diversity, RoV can cause recurrent infections with different genotypes. Repeat infections are associated with decreasing severity, with several studies estimating that at least two

RoV infections are sufficient to prevent further symptomatic infection^{7,8}.

Two live-attenuated RoV vaccines have been licensed for use in infants since 2006^{9,10}. The availability of these vaccines and improved patient care has halved the global number of deaths attributable to RoV^{2,11}. One of the currently licensed vaccines is Rotarix (GlaxoSmithKline, GSK), which is a monovalent formulation that comprises of the most dominant human genotype globally, G1[P8], and is recommended for use as a two-dose oral vaccine for infants between 6 and 24 weeks of age¹². Rotarix was developed using a RoV strain isolated from a human and has been shown to be safe and immunogenic in humans. Notably, despite Rotarix being monovalent, the vaccine has been observed to provide at least partial protection against other circulating genotypes, including the less common G9[P8]¹³. The alternative vaccine is RotaTeq (Merck), which is a recombinant bovine-human pentavalent formulation containing capsid proteins from the five most common disease-causing variants in humans (i.e. G1, G2, G3, G4 and P8)¹². RotaTeq is administered orally to infants in three doses at 2, 4 and 6 months of age. Both Rotarix and RotaTeq have been shown to not impede immunological responses against other standard Expanded Programme on Immunization (EPI) vaccines (and *vice versa*), when administered together^{14–16}.

The initial safety, immunogenicity and efficacy studies for Rotarix and RotaTeq were conducted in Europe and North America^{17,18}. Both Rotarix and RotaTeq were found to be highly immunogenic and efficacious in these settings, with significant protection observed against symptomatic RoV disease and severe forms of RoV gastroenteritis; vaccine efficacy against RoV disease and severe gastroenteritis for Rotarix was >85% and >95%, and for RotaTeq >70% and 98%, respectively^{17–19}. However, the high protective efficacy of these vaccines was not reproduced in LMIC, with studies in Bangladesh and Mali generating efficacies of 18% and 43%, respectively^{20–22}. A potential explanation for regional differences in RoV vaccine protection is human genetic variation, which may impede the generation of an appropriate protective immune response. Vaccine studies conducted in twins have estimated the genetic heritability of vaccine responses to be between 39 and 89%^{23–26}. It is only with recent advances in genomics and transcriptomics that we have been able to begin to identify these heritable traits²⁷. Recent genome wide association studies (GWAS) following vaccination with hepatitis B and smallpox or after natural infection have identified several single-nucleotide polymorphisms (SNPs) associated with antibody responses^{28–35}. Furthermore, early gene expression profiles have been described for several viral vaccines, including small pox, yellow fever, and influenza^{36–42}. Functional genomics or transcriptomic studies were able to correlate early gene expression following yellow fever or influenza vaccination with resulting T-cell and antibody responses^{43,44}. Genomics and transcriptomics now permit the investigation of complex biological processes following vaccination and the identification of new correlates that may predict protective vaccine responses. Despite

the importance of human genetics in vaccine-induced immune responses, GWAS and gene expression studies have yet to be conducted following RoV vaccination in infants.

Rotavirus vaccination in urban Vietnam

Acute diarrhoeal disease remains a leading cause of morbidity and mortality in children aged under 5 years in Vietnam^{45–48}. The community incidence of diarrhoeal illnesses in infants (<1 year of age) in southern Vietnam is estimated to be 271 per 1,000 infant-years, of which >50% is RoV infection⁴⁶. Both the Rotarix and RotaTeq have been tested and are licensed in Vietnam, but are not yet incorporated into the national immunization program^{20,49}. These vaccines induced incomplete protection when tested in Vietnamese populations, where immunogenicity studies observed seroconversion to RoV-specific IgA in only 63.3% (95% CI: 54.3–71.6) of vaccinated Vietnamese infants⁴⁹. Furthermore, a phase III, double blind, randomized control trial in Nha Trang, Vietnam observed a RoV vaccine efficacy of 63.9% (95% CI: 7.6 to 90.9) against severe RoV gastroenteritis²⁰. The circulating strains (G1[P8] and G3[P8]) in that region of Vietnam at the time of the vaccine trials were similar to those circulating in high-income countries where vaccine efficacy was >97.5%⁵⁰. Therefore, strain divergence is unlikely to explain the low vaccine efficacy in Vietnam. Differences in human genetics that underscore much of the variation in vaccine responses are also known to affect the immunogenicity and effectiveness of RoV vaccines^{51–53}. These differences in heritable traits may underlie the reduced immunogenicity of RoV vaccines observed in Vietnamese infants and account for lower vaccine efficacy.

In summary, as the leading cause of morbidity and mortality from acute gastroenteritis in children under five years, RoV is a pathogen of global public health importance. At present, there are two World Health Organization (WHO)-approved RoV vaccines available. However, the efficacies of these two vaccines vary greatly between different geographical regions. Large gaps exist in our understanding of the immune processes following RoV vaccination, preventing the discovery of meaningful correlates of protection and effective vaccine development.

Objectives of the study

In this study we will stimulate the human immune system using the licensed Rotarix RoV vaccine and study how differences in host genotype and gene expression influence immune responses and vaccine failure. We hypothesize that human genetic and transcriptional variation may influence long-term protection from RoV diarrheal disease following RoV immunization in infants. Hence, the primary objective of the study is to investigate the impact of human genetic and transcriptional variation on immunological responses and long-term protection following RoV immunization in a population of infants in Ho Chi Minh City (HCMC), Vietnam. The secondary objectives of the study are to assess temporal immunological responses following RoV vaccination, the function of maternally derived antibody in short-term immunity, and RoV genotypes associated with vaccine failure cases.

Methods

Study design

This study will be an open single arm, single centre, interventional study conducted in HCMC, Vietnam. We aim to recruit 1,000 infants aged between 8 and 9 weeks (2 months of age) to the study. These infants will receive two doses of Rotarix vaccine, with an interval of 28–37 days (or 4–5 weeks). The parents will be requested to bring the infants to the study centre 2–3 days following each Rotarix vaccination to receive the standard EPI vaccinations. The infants will then be under active surveillance for RoV-associated diarrhoea until the age of 18 months. Blood samplings will occur pre- and post-vaccination (at 2 and 3 months of age) and at routine visits at 6, 12 and 18 months of age (Table 1). A schematic diagram of the study is shown in Figure 1. Protocol version: Version 4.2, 3 January 2018.

Inclusion criteria

Male and female infants aged between 8 and 9 weeks (approximately 2 months) attending Hung Vuong Hospital (HVV) to receive their standard EPI vaccinations will be invited to join the study. The infants must also be currently registered residents of the district or neighbouring districts of HCMC where the main study site is located (districts 1, 5, 6, 8, 10, or 11), with no specific intention of relocating in the next 18 months. Parents or guardians of all participating infants must provide written informed consent for their child to be enrolled in the study and agree to comply with study procedures, including human genetic studies.

Exclusion criteria

Infants will be excluded from the study for any one of the below criteria:

- Refusal to consent by parent or guardian.
- Parent or guardian under 18 years of age.
- Premature birth (i.e. gestation period <37 weeks).
- Infants who have already been immunized with either a RoV vaccine or the standard 2-month EPI vaccinations.
- History of hypersensitivity to any components of the vaccine or adverse vaccine event.
- History of intussusception or congenital malformation of the gastrointestinal tract in the child that is likely to predispose child to intussusception.
- History of severe combined immunodeficiency disease (SCID), acquired immune deficiency syndrome (AIDS) or Human immunodeficiency virus (HIV) positivity, or other known immunodeficiency syndromes that may place the child at risk during immunisation.

Recruitment

Parents or guardians visiting HVV in HCMC, Vietnam with infants (between 8 and 9 weeks of age) for their check up and

Table 1. Sample types, time-points and purpose for collection throughout the study.

Sample No.	Infant age	Volume	Purpose
Infant Blood			
#1 ^a	8 – 9 weeks (Rotarix 1 st dose)	2 ml	Serology, DNA, & RNA-seq
#2 ^b	2–3 days post-1 st Rotarix dose	1 ml	RNA-seq
#3 ^c	12 – 14 weeks (Rotarix 2 nd dose, 28 – 37 days after 1 st dose of Rotarix)	2 ml	RNA-seq, serology
#4 ^d	2–3 days post-2 nd Rotarix dose	1 ml	RNA-seq
#5	26 weeks \pm 4 weeks (Approx. 6 months)	1 ml	Serology
#6	52 weeks \pm 4 weeks (Approx. 12 months)	2 ml	Serology
#7	78 weeks \pm 4 weeks (Approx. 18 months)	2 ml	Serology
Diarrhoeal stool Sample			
	Home visit	N/A	Screen for RoV (and other enteric pathogens)
	HTD visit	N/A	Screen for RoV (and other enteric pathogens)

^aBlood sample collected prior to the 1st dose of Rotarix.

^bBlood sample collected prior to the 2-month standard EPI vaccination.

^cBlood sample collected prior to the 2nd dose of Rotarix.

^dBlood sample collected prior to the 3-month standard EPI vaccination.

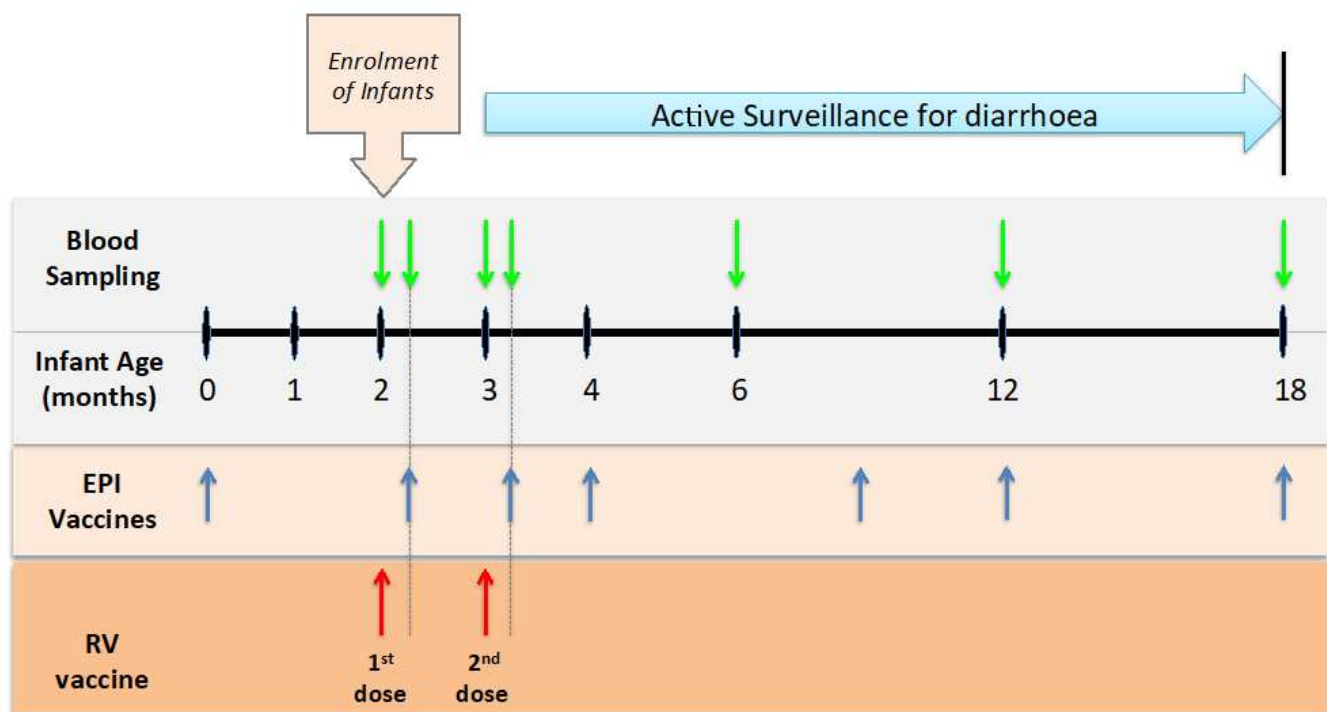


Figure 1. A schematic diagram showing the study design, enrolment and sampling strategy for the study. Infants will be enrolled to the study between 8 to 9 weeks (i.e. approximately 2 months) of age and will receive the two doses of Rotarix at 2 months and 3 months of age. Then the 2nd and 3rd month standard EPI vaccinations will be given 2–3 days following each dose of Rotarix. Scheduled blood sampling will occur prior to each Rotarix dose and prior to the 2nd and 3rd month EPI vaccinations. Further scheduled blood sampling and surveillance (both passive and active) for diarrhoeal events will continue until the end of the study when infant reaches 18 months of age.

EPI vaccinations will be referred to the study enrolment team if the infant fulfils all inclusion criteria. Recruitment activities will occur in the Pediatric Outpatient Clinic Department of Newborns of HVH only. A research nurse will explain the study, detail the risks and potential benefits, provide written information in the form of a brochure and invite the parents (or guardian) and infant to participate in the study. This study targets a vulnerable population, both in terms of the young infants and parents of the young infants. Therefore, following explanation of the study, sufficient time will be given for parents (or guardians) to make an informed decision whether to be involved. In all cases, written informed consent will be obtained prior to enrolment. The target sample size of 1,000 participants will be enrolled within an anticipated duration of 2 years. Following enrolment, a study nurse will ask the parent or guardian details on baseline demographic, residential location, socioeconomic, health status of infant (and mother), specific risk factors for acquiring diarrhoeal diseases (such as water usage, sanitation and hygiene habits, concurrent familial illness) and vaccine history of the infant.

Intervention

The RoV vaccine that will be used in this study is the Rotarix vaccine manufactured by GSK Biologicals, which is licensed for use in Vietnam. All participating infants will receive two doses of Rotarix vaccine following standard Rotarix immunization protocol (as detailed by manufacturer). The participating infants will receive the 1st and 2nd dose of oral Rotarix at 2 months (i.e. between 8 and 9 weeks) and 3 months (i.e. between 12 and 14 weeks) of age, respectively, with 28–37 days or 4–5 weeks between doses. If the infant regurgitates a majority of the vaccine dose, we will follow standard practice and give another dose of the vaccine. If the infant is hypersensitive to the 1st dose of Rotarix, then the infant will not receive the 2nd dose and will be excluded from further participation the study. The participating infant will be observed for at least 30 minutes after vaccination for any significant acute reactions, with appropriate medical treatment readily available in case of anaphylactic reaction. Any adverse events occurring during the observation period will be recorded. Parents and legal guardians will be instructed to contact the study team immediately should the infant manifest any signs or symptoms they perceive as serious or if the infant is admitted overnight to hospital. In the instance that the participating infant is experiencing any form of acute diarrhoea (i.e. mild, moderate or severe), vomiting or any other acute illness, vaccination will be delayed until at least 72 hours after resolution of symptoms. A reminder call will be given four weeks after the 1st dose to remind parents to bring their infant back to HVH for the 2nd dose of Rotarix to ensure the completion of the 2nd dose of Rotarix vaccine.

Blood sample collection and follow-up visits

Prior to receiving each dose of vaccine, the study nurses will collect a 2-ml blood sample from the participating infant; 1 ml of blood will be immediately transferred into an appropriate ribonucleic acid (RNA)-preservation blood tube for

transcriptomic analysis, and the remainder will be transferred into an ethylenediaminetetraacetic acid (EDTA)-containing blood tube for serology and genotyping. The parent or guardian will be requested to bring the infant for follow up visits 2–3 days after each dose of vaccine, and at 6, 12 and 18 months of age (Table 1). During these follow-up visits the study nurse will examine the infant, complete a questionnaire, collect a blood sample from the infant (1 ml at 2–3 days post-vaccination and at 6 months, and 2 ml at 12 and 18 months of age for transcriptomics and/or serology) and then give the infant the appropriate EPI vaccinations. The blood for RNA sequencing will be frozen, while blood collected for deoxyribonucleic acid (DNA) sequencing and serology will be stored at ambient temperature prior to being transported to the sample-processing laboratory within 24 hours of collection.

Passive and active surveillance for diarrheal events

A diarrhoeal event in this study will be defined as the occurrence of ≥ 3 non-formed stools (or ≥ 1 , if bloody) in a 24 hour period²⁶, and an increase in frequency, reduction in consistency or notable change in quality of the stools, compared to what the parent or guardian considers to be normal for the child²⁷. As part of the research activity, diarrhoeal events will be passively and actively surveyed to measure diarrhoea caused by RoV infection (despite RoV vaccination). Passive and active surveillance for diarrhoea will begin one week after the 1st Rotarix dose, and continue up until the study participant completes or reaches the end of the study. Procedure for passive and active diarrhoea surveillance will be conducted similar to a recently completed cohort study (Figure 2)⁵⁴.

In order to perform passive surveillance for diarrhoeal events in study infants, parents will be requested to alert a member of study staff when the child experiences an episode of diarrhoea through calling or texting a study nurse or doctor. Additionally, for active surveillance, a designated family member will be contacted weekly by phone (short message service [SMS] or call) inquiring if the enrolled child has experienced an episode of diarrhoea in the preceding seven days. If diarrhoea is confirmed by the family member, a study nurse will be made aware and they will call the family to arrange a mutually convenient time for a household visit. At the household, a short disease history research questionnaire will be administered, which will include details of illness onset, illness of close contacts, dehydration status and other clinical symptoms. The study nurse will clinically evaluate the child and advise as to whether hospital attendance is needed. Oral rehydration and electrolytes supplements will be provided to the family. Study nurses will be trained carefully to ensure that patients seek treatment when necessary. The nurse will provide clinical advice and will recommend whether the parent seeks standard of care at a local healthcare facility. At the household visit, the study nurse will collect a stool sample from the child if a specimen is available at the time of visit. If no sample is available at the visit, the study nurse will give the family a sterile container and will arrange for collection and transport within 24 hours.

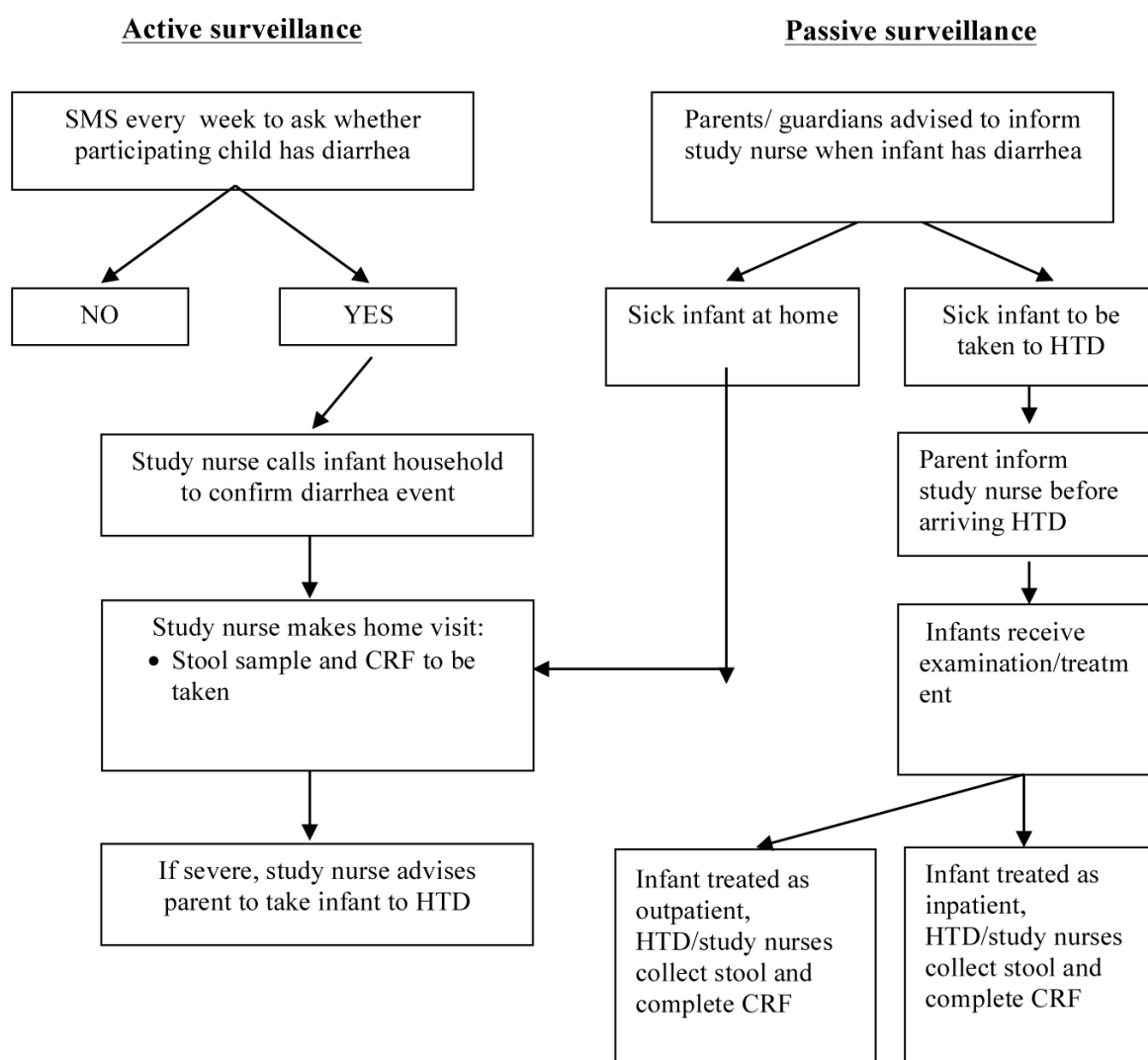


Figure 2. Flowchart showing the strategy for active and passive surveillance of diarrheal events in the participating infants following RoV vaccination up until end of study at 18 months of age.

If an infant experiences an episode of diarrhoeal disease that the parent feels is severe enough to seek medical care, we will request the parents to alert one of our study nurses and to consider attending Hospital for Tropical Diseases (HTD) in District 5, HCMC for consultation. If the family attends HTD for diarrhoeal illness, they will be requested to call a study nurse or doctor prior to their visit to alert study staff that they are coming. Following clinical examination of the infant, the study nurses will collect a stool sample and complete a study questionnaire. The infant will then be treated with standard care for diarrheal disease.

Adverse event (AE) and serious adverse events (SAEs)

On the days of Rotarix administration and 2–3 days later, the study nurse will report any observed adverse events. The

participant's parents or guardians will also be asked to report any observed adverse events during their post-vaccination visits (2–3 days post-vaccination) and follow-up visits (at 6, 12 and 18 months of infant age). Furthermore, the participant's parents or guardians will be urged to immediately report any SAE or suspected unexpected serious adverse reactions (SUSARs) to the study team immediately. For SAEs, investigators will notify the Oxford University Clinical Research Unit (OUCRU) Clinical Trials Unit (CTU) within 24 h. SAEs and SUSARs will be notified to the OUCRU CTU until trial closure. CTU will perform an initial check of the report and request any additional information if required and ensure review by an independent medical monitor. Furthermore, CTU will report all SAEs to the Ministry of Health (as is the regulation for clinical trials) and to GSK, Vietnam. Since Rotarix is a licensed vaccine and

proven to be safe for use in infants, the parents will not be asked to keep a diary card to closely monitor and record all adverse reactions.

On-site monitoring

A site initiation visit will be conducted for each study site by staff from the OUCRU CTU. All essential site staff including the PI, lead pharmacist and lead research nurse must be in attendance. The initiation training will include training in the vaccine administration and practices, as well as the trial procedures. Monitoring will then be carried out approximately annually at each site by OUCRU CTU staff. The monitors will require access to all participant medical records defined as source document. The investigator (or delegated deputy) should work with the monitor to ensure that any problems detected are resolved.

Geospatial mapping

Residential household addresses of the participating infants will be obtained from parents and guardians. Then geographical co-ordinates of their residence will be ascertained using the residential household addresses for the purpose of descriptive and correlational spatial epidemiology. The coordinates will be entered into a geographical information system (GIS), such as ArcView (ESRI Inc., Redlands, CA, USA). To protect patient confidentiality all maps arising from this research will display data at low resolutions in efforts to prevent identification of individual households.

Laboratory methods

Blood Samples: Whole blood from the EDTA tubes will be separated into blood cells (red cells and buffy coats) and plasma. Human DNA will be extracted from blood cells and stored at -20°C until required for genotyping. The plasma will be aliquoted into cryo-vial tubes for storage at -20°C until required for serology. RoV-specific antibodies in plasma will be assessed using conventional RoV enzyme-linked immunosorbent assay (ELISA)^{55–57}. Blood will be collected into blood RNA tubes (which lyses cells and preserves RNA) and then stored at -80°C until RNA extraction for transcriptomic studies.

Stool samples: All stool samples will be screened for enteric parasites, bacteria and viruses using established laboratory methods, for example multiplex real-time polymerase chain reaction (PCR) and/or the xTAG gastrointestinal pathogen panel (Luminex Molecular Diagnostics, Austin, TX, USA). The xTAG multiplex assay will screen stool samples for enteric pathogens, including RoV A, *Salmonella* species (spp.), *Shigella* spp., *Campylobacter* spp., *C. difficile* (toxins A and B), enterotoxigenic *E. coli* (ETEC) heat labile and heat stable enterotoxins, *E. coli* O157, Shiga toxin-producing *E. coli* spp., *Vibrio cholerae*, *Yersinia enterocolitica*, fecal adenovirus 40/41, norovirus GI/GII, *Giardia* spp., *E. histolytica*, and *Cryptosporidium* spp.⁵⁸. The remaining stool sample rest will be stored at -80°C until further use. In samples that test positive for RoV, we will conduct whole genome sequencing of RoV using a recently developed a sensitive sequencing method that can

successfully sequence RoV genomes from clinical faecal samples with over >90% genome coverage⁵⁹. Briefly, this technique involves antibody capture of whole rotaviral particles from faecal samples and subsequent amplification with whole RoV genome sequencing (Illumina MiSeq).

Human genetic studies

For genetic studies, extracted anonymised-linked DNA will undergo genotyping using a DNA microarray or sequencing⁶⁰. For transcriptomics studies, RNA will be extracted from the anonymised blood RNA tubes using standard methods⁶¹. Standard quality control procedure will be undertaken to assess the suitability of the isolated RNA for sequencing, including (but not limited to), the assessment of RNA integrity and concentration. RNA that passes these quality control checks will then undergo library creation for sequencing. This library creation will include protocols to deplete globin RNAs and select polyadenylated messenger RNAs, to maximise sequencing coverage of RNAs from white blood cell types. The RNA libraries will then be sequenced and analyzed.

End of study

Participation in the study will end once the study participants have completed the follow-up visit at 18 months of age. Lost-to-follow-up cases are defined as those participants that the study team are unable to reach after a minimum of three documented attempts over a period of two months after a missed visit. The study participants may withdraw consent and all further study contacts at any time when their parents or guardians decide. The study participants will be withdrawn by the chief investigators if found not to meet inclusion or exclusion criteria or the study requirements after starting the study.

Outcome measures

The primary outcome will be Rotarix vaccine failure events (i.e. symptomatic RoV infection) from the first dose of vaccination to 18 months.

The secondary outcomes will include:

Quantification of the antibody response following immunization. The temporal kinetics and properties of RoV-specific antibody responses following immunization will be used to investigate the effect of human genetics, maternally transferred antibodies, and geospatial and epidemiological factors that may impact on humoral response following vaccination. An assessment of infecting RoV genotypes in vaccine failure cases will be performed to investigate whether vaccine failure cases are a result of varying RoV genotypes.

Sample size justification

A recent study conducted passive surveillance for diarrhoeal cases and estimated the incidence of diarrhoeal cases in Vietnam in infants during their first year of life as 271 cases per 1,000 infant years⁴⁶. However, an active surveillance study for diarrhoea in Vietnam identified twice as many diarrhoeal cases as in passive surveillance⁶². Therefore, the true incidence of diarrhoea in Vietnam is approximately 540 cases per 1,000

infant years. The aetiological agent of 53% of the identified diarrhoeal cases was RoV⁴⁶, which estimates the average incidence of RoV in Vietnam to 286 cases per 1,000 infant years. Since the proposed study follows each participant for 18 months, we would expect to observe 429 cases per 1,000 infants (i.e. 286 cases x 1.5 years) during the study period. Phase III RoV clinical trials in a Vietnamese cohort observed 64% vaccine efficacy within the first two years after vaccination²⁰. Using a vaccine efficacy of 64%, the incidence of RoV vaccine failure cases can be approximated to 155 diarrhoeal cases per 1,000 infants.

Although not established as a correlate of protection, high titres of RoV-specific antibodies are associated with protection in vaccine efficacy studies (risk ratio=0.5)⁶³. Investigating associations between humoral immunity and RoV vaccine failure events is an important aspect of this study. Using a risk ratio of 0.5 for high RoV-specific antibody titres (i.e. >90 U/ml), we can calculate the probability of a vaccine failure event in high antibody titre group as being 7.8% (while 15.5% for antibody titres lower than 90 U/ml). Using a power of 80% we estimate a minimum of 144 vaccine-failure events are required to observe a difference at 95% confidence level. A conservative recruitment of a sample size of 1,000 infants will lead to 155 vaccine-failure events, which is above the minimum vaccine-failure events required to power the study at 80%.

Analysis

Epidemiological, antibody and geospatial analyses. The primary endpoint, i.e. the proportion of vaccine success in protection against diarrheal disease caused by RoV, will be estimated by the number of individuals who do not experience a diarrheal episode caused by RoV after vaccination and until 18 months of age. Descriptive analyses will be performed to explore the variation in geospatial, demographic, socioeconomic factors and antibody titres between infants with RoV associated diarrhoea (aka vaccine failure events) and infants protected from RoV associated diarrhoea. Statistical analysis will be conducted using parametric and non-parametric tests; Chi-square or Fisher's exact test will be used for categorical variables and Student's t-test or Kruskal-Wallis tests for continuous data, respectively.

Genetic association and transcriptomic analyses. Integrating genome-wide genotype data with serial measurements of the transcriptional and humoral responses to RoV vaccine in a longitudinal study should allow us to identify genes that influence vaccine responsiveness and subsequent immunity to RoV. Genotypes will be called using genotype calling software packages for microarrays (e.g. optiCall)⁶⁴, or variant calling pipelines for whole-genome sequencing data (e.g. GATK)⁶⁵. To assess variants that are not on the genotyping array, imputation and phasing will be conducted using imputation software (e.g. PBWT) using a publicly available reference panel (e.g. 1,000 Genomes, or Haplotype Reference Consortium panel). The reference panel will be augmented in the Human leukocyte antigen (HLA) region by HLA sequencing of a subset of the study cohort (e.g. [Histogenetics](#)). We will also use the SNP data to accurately impute ABO blood groups and secretor status.

Standard sample and marker quality control procedures will be applied. Quantitative trait loci (QTL) models will be built to detect associations between genetic variation and variation in gene expression. Linear regression-based association models will also be built using response variables including antibody titres and cases of vaccine failure.

The quality of sequencing reads will be evaluated (e.g. using FastQC) and poor-quality reads/adaptor contamination will be filtered out or trimmed (e.g. cutadapt, Trimmomatic). Reads from ribosomal RNA or non-human contamination will also be filtered out (e.g. SortMeRNA). Reads will be aligned to the human reference assembly (e.g. TopHat, STAR) and alignment quality will be evaluated (e.g. QualiMap). Transcript abundances will be quantified (e.g. Salmon, kallisto) and batch effects detected and removed (e.g. EDASeq, PEER, sva). Transcript abundances will also be assessed for other biases such as length or GC bias. Transcripts (or sets of transcripts) that are differentially expressed (or differentially spliced) between experimental groups (e.g. time points or high vs. low responders) will be identified (e.g. DESeq, edgeR, limma/voom), with appropriate covariates (e.g. gender) included in the model. To increase statistical power and biological interpretability, transcripts will be grouped into sets such as co-expression networks or transcriptomic modules. Existing modules generated in previous systems vaccinology studies will also be leveraged, and new modules will be generated and annotated (e.g. using gene set enrichment analyses based on Gene Ontology/pathway databases). From these differentially expressed/spliced transcripts (or modules), we will aim to identify signatures that correlate with and/or predict important endpoints such as antibody titre or vaccine failure. We will also investigate the utility of computational deconvolution methods for obtaining immune cell subtype-specific gene expression profiles from bulk expression data, in order to conduct analyses in a cell-type specific manner.

Best practices in the field of statistical genetics (both genetic and transcriptomic) are constantly evolving, hence computational analyses will be adapted to include improved tools and pipelines as they become available. We will also develop statistical methods for modelling multi-omic studies with longitudinal sampling.

Data management and storage

Participant data collected at HVH site and during home visits will be recorded using laptops and tablets directly on to electronic case report forms (eCRFs) on a secure password-protected electronic database. Electronic data entry devices will be password protected and accessible only by authorised users. Any information collected on the laptops or tablets will be sent immediately to the secure OUCRU server and no information will be retained on the devices. Paper CRFs will be used during diarrhoeal illness presentations at the HTD study clinic and study nurses will transfer data from paper CRFs onto the password-protected electronic database. Paper CRFs will be provided to the CTU at OUCRU, who store them in long-term storage in the restricted storage facility at the HTD. Laboratory data from anonymised-linked (i.e. identified by an enrollee identifier only) pathogen screening will be recorded on

the standard study laboratory forms and subsequently input into a secure password-protected electronic database by a member of the study team.

The access control restrictions for this data are as follows: All systems (including computers, storage systems, portable devices, and the users and administrators who control that equipment) which are used to store, process, or transmit unencrypted data must limit access to authorised individuals only, who are members of an access control group designated by the WSI Principal Investigator (Anderson) and to WSI systems administrators. Data stored on (or transmitted via) systems that do not implement the required access control restrictions will be suitably encrypted.

The present clinical trial will not involve a data monitoring committee (DMC) since the interventional product, Rotarix, being given to infants is licensed and the safety profile is well characterized.

Participant confidentiality

Parents/guardians will be assured that all information generated in this study will remain confidential. The names and addresses of study participants will be recorded in the database but kept separate from the main body of data and access will be strictly controlled through password protection. Any scientific publications or reports will not identify any child by name or initials. When the research team reviews their notes, they are also bound by professional confidentiality. All genetic data resulting from this study will be de-identified using current best practices. Anonymized DNA and RNA sequence data will be archived indefinitely in the European Genome-phenome Archive (EGA) and shared with other researchers around the world *via* a managed access system.

Data sharing

In line with research transparency and greater data access, this trial is registered at ClinicalTrials.gov and a data sharing policy is in place. This policy is based on a controlled access approach with a restriction on data release that would compromise an ongoing trial or study. Data exchange will comply with Information Governance and Data Security Policies in all of the relevant countries. In order to protect participant privacy and confidentiality, only anonymised data will be shared with collaborators. Anonymised DNA and RNA sequence data will be archived indefinitely in the European Genome-phenome Archive (EGA), a research database managed by the European Molecular Biology Laboratory (EMBL) European Bioinformatics Institute (EBI), Hinxton, Cambridge, UK. Anonymised genetic data relating to human research participants will be stored according to the WSI Human Data Security Policy. Data for this project will fall into the WSI Data Security Level 2 category.

Publications

No aspect of this study will be reported without approval from all principle investigators. In line with Wellcome Trust policy that the results of publicly-funded research should be freely available, manuscripts arising from the trial will, wherever

possible, be submitted to peer-reviewed journals which enable Open Access via UK PubMed Central (PMC) within six months of the official date of final publication. All publications will acknowledge the trial's funding sources.

Ethics approval and consent to participate

The current study has received ethical approval from the Oxford Tropical Research Ethics Committee (OxTREC, study reference 56-16), the internal review board (IRB) of Hung Vuong Hospital (HVH), the IRB of Hospital for Tropical Diseases (HTD) and from the ethics committee in biomedical studies at the Ministry of Health in Vietnam. Any future protocol amendments prior to implementation will require review by ethical committees of HVH, HTD and OxTREC. The present trial is also registered at ClinicalTrials.gov (NCT03587389). Parents or guardians of all infant participants will provide written informed consent prior to participation of infant in the study.

Discussion

Diarrheal disease caused by RoV is still the leading cause of morbidity due to gastroenteritis in children under 2 years of age globally^{2,46}. Two RoV vaccines are licensed for use in humans and have shown high efficacy against RoV-caused gastroenteritis in western countries, but poor efficacy has been observed in parts of Africa and Asia, including Vietnam^{13,16,17,20}. It is unclear why RoV vaccine has such variable efficacy in different parts of the world. Since vaccination is currently one of the only methods of infection control, it is imperative that we understand the cause of RoV vaccine failure and work towards improving its efficacy. This study aims to investigate host factors as a possible explanation for differences in immune responses and efficacy of RoV vaccines.

Numerous studies have identified the role of human genetics and gene expression in dictating immunological responses (including humoral and cellular responses) to natural infection and vaccine response^{23,28,31,41,44,66}. Technological advances now allow us to screen entire genomes and transcriptomes of individuals faster and at an economical cost⁶⁷. Therefore, the current study takes a systems biology approach to investigate the host genetic and transcriptomic factors influencing both immunological responses and RoV vaccine failure (versus success) in the Vietnamese population. This study will be the first systems biology investigation for RoV vaccines and also the first such study conducted within the Vietnamese population. A major strength of the study is the large sample size will allow us to power the study to investigate the contribution of host genetic and transcriptomic factors to an epidemiologically relevant clinical end point, i.e. gastroenteritis due to RoV vaccine failure events. The genetic and transcriptional factors required for humoral responses to RoV will also be deciphered in the current study. Furthermore, active and passive surveillance for diarrhoea following vaccination will allow us to sequence the viral genomes of breakthrough RoV infections. However, due to the absence of a no vaccination control group, we will be unable to assess the true diversity of RoV circulating during the study. If the current study is successful in identifying both genomic and transcriptomic factors that are associated with protective vaccine responses, then we can foresee

these findings being utilized to design more immunogenic vaccines or more personalized vaccines to different populations of individuals.

Trial status

The trial began with the recruitment of the first participant on 19th March 2019.

Data availability

Underlying data

No underlying data are associated with this article.

Extended data

Zenodo: The influence of human genetic variation on early transcriptional responses and protective immunity following immunization with Rotarix vaccine in infants in Ho Chi Minh City in Vietnam: A study protocol for an open single-arm interventional trial. <https://doi.org/10.5281/zenodo.4075332>⁶⁸.

Appendix A contains the World Health Organization Trial Registration Data Set.

Reporting guidelines

Zenodo: Appendix B contains the PRISMA-P checklist for 'The influence of human genetic variation on early transcriptional responses and protective immunity following immunization with Rotarix vaccine in infants in Ho Chi Minh City in

Vietnam: A study protocol for an open single-arm interventional trial'. <https://doi.org/10.5281/zenodo.4075332>⁶⁸.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Author contributions

Study concept, design and methodology was by SB, CA, RdA, and BYHB. Ethics process, project management, administration and study implementation lead by TMP with assistance from RdA, NLTQ, PTTT, TTND, NTTN, CV, HVH, LTHN, HTTT, LPT, VTND, HTNT, NPTKT, NNH, MCD, TDH, TTTP, TAD, LD, NTHN, NTE, NTTH, LTH, TCD, GET, EK, LLV, BTTT, HTDT. Funding for study acquired by SB and CA. Manuscript prepared by SB, RdA, TMP, and read and approved by all authors.

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? Julie Bines 

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This protocol has been registered on clinicaltrials.gov in July 2018 and started recruitment in March 2019. Therefore the comments on this study design and approach to the research questions defined I appreciate will likely have limited impact on the conduct of the study. While the intention of the study is novel, the ability to achieve the stated outcomes based on the information presented presents some concern. These are summarised below:

1. Clarification about the primary and secondary endpoints. There is confusion with key endpoints on page 5 and page 9. If the primary endpoint is "vaccine failure" events then this seems primarily a vaccine effectiveness study.
2. Antibody responses to be used to determine the secondary outcomes are not defined. Presumably this is anti-rotavirus serum IgA, but there is confusion on page 5 on implying that maternally derived antibodies are to be compared. As there is a primary interest in immune responses and genetics there seems a missed opportunity to look more broadly at immune responses including innate and adaptive immune responses. If serology is for sIgA seroresponses – this needs to be defined (i.e. is this seroconversion post vaccination when compared to a baseline prevaccination blood or an increase above a threshold?). For most studies assessing immune response for Rotarix the primary outcome is on the 28 day post dose 2 blood using a >3 fold rise from baseline (for seroconversion) or >20 for a sero-response – noting that in this setting some infants may have a sero-response at baseline. Since there is no blood draw at this timepoint it is difficult to know how the results of this study will compare with previous studies in this and similar environments. Where is analysis to be performed?
3. This paper seems to have some out of date information and needs to be revised with respect to new data, for example:
 1. Page 4 all children have a rotavirus infection by 5 years is from a publication prior to rotavirus vaccines – now in >100 country NIP. Appreciate that this may be still true in Vietnam.
 2. There are 4 rotavirus vaccines now WHO prequalified not 2 (page 2 and 4) with

- updated data on the effectiveness of these vaccines across may settings.
3. It is not correct to say that it is unclear why vaccines have less efficacy in LIMCs – there is a consensus that a number of factors may contribute to a greater or less extent in different settings.
 4. Update the data on rotavirus vaccine uptake in the private market including the locally licensed and manufactured Rotavin vaccine.
4. There is no biological hypothesis provided to why genetic and transcriptional information may impact responses, and whether there is sufficient variation within the study population to identify if there are differences. Is it likely that any differences identified might have a broad role of immune responses and not just to rotavirus vaccines? It would seem reasonable to measure immune responses to the other EPI to determine this.
5. There is no reference to the considerable data on histoblood group antigen status which does have a solid basis for genetic influence rotavirus vaccine responses based its the role as a receptor for rotavirus in the gut.
6. There are concerns about the calculation of the sample size. The investigators quote vaccine efficacy data from the Phase III Rotarix study of 65% as a basis for their estimation. However this is based on reduction in severe rotavirus gastroenteritis episodes (Vesikari score >11). The impact of vaccines on mild or moderate rotavirus gastroenteritis is lower in both LIMCs and high income countries. This study does not seem to make a distinction between severity of episodes in their analysis or calculation of SS. The characteristics of the immune responses will likely be very different in severe and mild episodes so this will provide a challenge in interpretation of data.
7. No information is provided on the EPI vaccines which will also impact immune responses and/or vaccine take of Rotarix (as is the case for OPV).

Is the rationale for, and objectives of, the study clearly described?

Partly

Is the study design appropriate for the research question?

Partly

Are sufficient details of the methods provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Rotavirus vaccinology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have

significant reservations, as outlined above.
